Tubulointerstitial fibrosis

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Objectives

- Discuss the relationship between interstitial fibrosis and renal outcome
- Discuss the possible mechanisms by which glomerular disease may induce interstitial lesion
- Discuss the changes that occur in collagen regulation during interstitial fibrosis
- Discuss relationship of TGF-*B,* and Hsp 47 to interstitial fibrosis
- Discuss current treatments of interstitial fibrosis

Real Quiz

- What is best predictor of renal outcome in glomerular disease?
- A. The sclerotic index
- B. Degree of proteinuria
- C. Age of patient
- D. Degree of tubulointerstitial damage

Changes in collagen homeostasis induced by TGF-*B*

- A. Increase in MMP activity, decrease in TIMP activity?
- B. Increases in collagen 1,III, and MMP?
- C. Increase in collagen III, and decrease in MMP and collagen IV and increase in TIMP?
- D. Increase in collagen I, III, IV,MMP, and TIMP?

Compare the Ace inhibitors and AT2 receptor antagonists on the tubulerointerstitum?

- A. Both reduce TGF-*B* completely ?
- B. Effects are likely independent of antihypertensive effects?
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Relationship of TI disease to glomerular disease

- Progressive tubulointerstitial fibrosis is thought to be the common final pathway of all forms of chronic renal failure
- Well established that the degree of tubular interstitial injury is best predictor of renal outcome.

Schiank,et al

- Studied histologic changes in 59 patients with a wide variety of glomerular disease
- Found inverse relationship between GFR and the degree of interstitial injury
- Validated in other studies that correlated serum creatinine to morphometric quantification, and the number of inflammatory cells

Tubulointerstial disease in FSGS

- Wherman,et al (Clin Nephrology 33: 115-122, 1990.
- Retrospectively looked at biopsy specimens of 250 FSGS patients and found that tubulointerstitial disease was best predictor of serum creatinine.

Mechanisms of tubulointerstitial injury associated with glomerular disease

- Multifactorial, and not clearly defined
- Possible mechanism include:
- Phosphate deposition
- Hyperlipidemia
- Proteinuria
- Active cytokine secretion by tubular cells

Proteinuria association with TI disease

- Tubular absorption of protein leads to lysomal degredation
- Albumin absorption leads to production of a chemotactic lipid
- Complement excretion and protein degradation induces complement activation in tubules
- Direct absorption of cytokines, GBM matrix material
- Absorption of transferrin produces hyroxyl ions

Mechanism not related to proteinuria

- Glomerular cytokines migration to interstitium and recruit inflamatory cells
- Injury uncovers antigenic cells such as heat shock proteins
- Tubular ischemia
- Generation of reactive oxygen species
- Direct interstitial generation of cytokines

All roads lead to Seoul

- Regardless of initial mechanism, chronic injury will induce interstitial fibrosis
- Increased production of cytokines, growth factors
- Upregulation of fibroblasts
- Change in quantity and quality of collagen production

Negative feedback

- Once fibrosis has been initiated there is progressive renal damage
- There is obliteration of postglomerular capillaries by fibrosis
- Continued production of fibroblast growth factors
- Amplification of immune injury

Therapeutic Intervention site

- Since the fibrosis appears to be irreversible after its initiation
- Interstitial fibrosis seems to be common end pathway for several glomerular diseases
- FSGS is induced by several entities
- Prevention of fibrosis prior to its initiation may be ideal area for a therapeutic intervention

First some definitions

- Collagen I,III early mesenchymal collagens that form bundles and thin fibrils.
- Fibronectin formed early with collagen I and III
- Together they form interstitium lattice
- Collagen IV formed later and associated with basement membranes
- Metalloproteinase (MMP) enzymes that degrade matrix material
- Tissue inhibitor of MMP (TIMP) inhibits MMP, thus decreases collagen degradation

Mechanism of tuberulointerstitial fibrosis

- Basically extracellular matrix deposition > then degradation.
- Phase 1 Induction (Activation of macrophages and cytokine release), recruitment of immune cells, recruitment and proliferation of fibroblast
- Phase II matrix deposition, with increase in collagen I, III, IV, fibronectin, and decrease in MMP activity usually modulated by increased TIMP activity. Maintenance of chronic inflammatory state.

Important Markers/Cytokines of tubulointerstial fibrosis

TGF-*B*

- Cytokine that participates in wound repair, growth
- Produced by both intrinsic renal cells and infiltrating macrophages
- Thought to be the major cytokine involved in extracellular matrix deposition in several organs

Up regulation is associated with several renal diseases

- Chronic transplant nephropathy
- Rat models of FSGS
- HIV nephropathy
- ADPCK
- Diabetic nephropathy
- Renal ischemia

Kopp,et al JASN 1999

- Alb/TgF-*B1* Transgenic mice develop glomerulosclerosis and proteinuria at 3 weeks
- 5 weeks noted to have increased amounts of collagen I,III in interstitium, mesangium, and capillary loops. No change in collagen IV levels
- Increased activity of TIMP>MMP

Hsp 47 “Colligin”

- Hsp 47 is molecular chaperone for collagen biosynthesis
- Thought to be expressed by altered myofibroblasts associated with collagen production,and plays a role in the secretion of procollagen from the endoplasmic reticulum
- Cells that can’t produce colligin also can't produce procollagen I mRNA
- Upregulated by TGF-*B 1*

Hsp 47

- Upregulated in several models associated with fibrosis
- Obsructive uropathy
- Antithymocyte induced glomerulonephritis
- Gentamycin induced nephropathy
- Bleomycin induced pulmonary fibrosis

Moriyama,et al KI Supp 1997

- Unilateral ureteral obstructed rats given either TCV-116, a AT2 receptor antagonist, or Lisinopril
- Hsp 47 upregulated in controls in 12 hrs, collagen I mRNA increased in 4 days
- Hsp 47 and collagen I mRNA reduced 65% 7 days after obstruction.

Pirfenidone

- 5-methyl-1-phenyl-2(1H)-pyridinone
- Novel new antifibrotic agent
- Mechanism of action is unclear

Pirfenidone Studies

- Initially used in bleomycin induced lung fibrosis, with dramatic decrease in lung hydroxyproline.
- Down regulation of ICAM on cultured synovial fibroblasts
- Reduced fibronectin synthesis in retinal pigment cells

Shimuzu, et al KI 1998

- Pirfenidone given to rats with unilateral urinary obstruction for 21 days
- Pirfenidone decreased the amount of kidney hydroxyproline content, especially if started prior to injury. No effect on sham control group
- Reduced TGF-*B* ,collagen type IV>1 mRNA, no effect on MMP-2 or blood pressure

Shimizu,et al. KI 1997

- Pirfenidone given to 5/6 nephrectomy rats two weeks after renal ablation
- Pirfenidone decreased renal hydroxyproline content, Type I and IV collagen, and TGF-*B*
- No statistical difference in proteinuria
- Pirfenidone attenuated the decreases in creatinine clearance in partial nephrectomy group.

Kopp,et al

- Alb/TGF-*B1* mice treated from 2-6 weeks of age with pirfenidone 800mg/kg/day.
- Substantially reduced the BUN, Creatinine, proteinuria, and tubulointerstitial disease
- No effect on normal controls treated with pirfenidone

Ace inhibition Marinides et al, KI 1990

- Used puromycin induced nephrosis (FSGS model)
- Treatment groups included Enalapril, and low protein diet
- Showed decrease in proteinuria as well as tubulointerstial lesions at 18 and 24 weeks

Ishidoya,et al. KI 1996

- Treated rats with unilateral obstruction with Enalapril 4-10 days after insult
- Showed decrease in interstitial volume, TGF-*B,* TIMP-1, collagen IV
- This study showed that delayed treatment with Enalapril can slow the progression of interstitial fibrosis.

Klahr and Morrisey KI 1997

- Used a unilateral obstructed rat model
- Treatments groups included 10 days of Ace inhibition, AT-1, AT-2 receptor blockade
- Noted Ace inhibition was superior to AT-2 receptor antagonist in decreasing interstitial volume,monocyte counts, TGF-*B* formation
- Ace inhibition thought to be superior secondary to kinin and nitric oxide production
- Other studies have shown equal benefit

Peters, et al

- Used rat Thy1.1 GN model treated with Ace inhibition, Lorsatan, or combination therapy
- Ace inhibition and Losartan groups reduced TGF-B by 45%, also reduced collagen markers
- TGF-*B* not further reduced at higher doses of either drug.Combination therapy did not reduced levels better than single agents
- Indicates that drugs with other mechanisms used in combination with Ace may reduce TGF-*B* further
- Blood pressure unchanged in treatment groups indicating that effect may be independent of HTN

HMG Co A reductase inhibitors Harris,et al

- Puromycin nephrosis model
- One group treated with Lovastatin, the other was treated with vehicle for 63 days
- Noted decrease in sclerosed glomeruli and tubululointerstial interstitial disease
- Also reduction in BUN/creatinine but no difference in proteinuria

Kim,et al KI 1995

- Human fetal mesangial cells stimulated with fetal bovine serum which induces production of monocyte chemotatic protein(MCP-1)
- One group incubated with Lovastatin, the other with controls
- Lovastatin inhibited mesangial cell production of MCP-1, and Mevalonate completely reversed the effects of Lovastatin
- Indicates that Lovastatin may have antifibrotic activity outside of its antilipid effects

Hyperlipidemia and progression of renal disease

- Again data concerning interstitial disease is based on animal studies
- Noted that lipogenic diets can led to FSGS with associated TI disease in several animal models
- Generally the extend of injury correlates with cholesterol level
- Seems to be independent of triglyceride level

Steroids

- Generally a lack of data in animal models of chronic interstitial fibrosis
- Fujiwara (Virchows 1984) amelioration of proteinuria with methylprednisolone in PAN nephrosis, however used model of where proteinuria spontaneously resolves in 20 days
- Dexamethasone has reduced levels of HSp 47

Nakamura, KI 1991

- Used variant of puromycin nephrosis rat model
- Treated with methylprednisolone for 20 days
- Showed amelioration of PAN induced changes in collagen IV but not in collagen I and III

Sesso,et al Ann Inter Med 1992

- Studied 37 Brazilian patients (some adults, some children) whose diagnoses included FSGS, MCD, and MPGN
- Tested B2M, retinol binding protein (RBP) prior and after course of steroids
- Higher levels of pretreatment B2M, and RBP were significantly higher in steroid unresponsive group
- Conclusion was severe tubular interstitial disease was a marker for steroid resistance

Other modalities
Probucol
Hirano, Nephron 1992

- PAN nephrosis model given either vehicle or probucol for 10 weeks
- Significant reduction of proteinuria, and increase his serum albumin vs. other lipid protocols
- No significant reduction in creatinine or BUN
- Significant reduction in glomerular and interstitial lesions

Probucol as antioxidant Lee, et al KI 1997

- PAN model studied for 32 weeks
- Treatments included probucol, or Vitamin E, given with normal or high cholesterol diet
- Therapy with probucol reduced proteinuria,

Mycophenolate Mofetil Fujihara,et al KI 1998

- 5/6 renal ablation model
- 4 groups (sham, sham +MMF, MMF with ablatation, and control ablation group) observed 60 days
- No differences in renal hemodynamics or hypertrophy, MAP,GFR, or proteinuria in all 4 groups
- 75% in interstitial expansion, and equivilant reduction in CD3 expression

- No change in amount of proteinuria

Our Protocol

- Rat puromycin model
- Studying the effects of Enalapril + Lovastatin, Pirfenidone, and the combination of all three medications
- End points are creatinine, proteinuria, lipids, histology, Hsp 47, and TgF-*B*
- Hoping to determine if pirfenidone is effective in FSGS, and combination therapy is more effective than current therapies

Post Test

- What is best predictor of renal outcome in glomerular disease?
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